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Full Paper

Characterisation of chitosan solubilised in aqueous formic and acetic acids

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Abstract: The intrinsic viscosity of chitosan (MW 7.9 x 10^5 g mol^{-1}) having a high degree of deacetylation and solubilised in aqueous formic and acetic acids was determined at room temperature. Contact angle and conductivity of the chitosan solutions were also studied. The values of critical coagulation concentration (CCC) were then obtained from the plots of contact angle or conductivity versus concentration.

Keywords: chitosan, intrinsic viscosity, contact angle, conductivity, critical coagulation concentration

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Introduction

Chitosan is an amino-sugar-containing polysaccharide usually obtained by alkaline deacetylation of chitin from crab and shrimp shells [1-2]. This fibril biopolymer is composed of β-(1$\rightarrow$4)-2-amino-2-deoxy-D-glucopyranose units (glucosamine units). It is a non-toxic, biocompatible and biodegradable polymer [3-4]. It has been widely used in diverse fields ranging from waste management to food processing, medicine and biotechnology [5-11].

The physical [12], chemical [13] and biological [14-15] properties of chitin and chitosan depend mainly on two parameters: degree of deacetylation (DD) and molecular weight distribution, both of which are affected by the source of chitin and the method of preparation. The DD also plays a significant role in affecting the molecular weight of chitosan. A lower DD leads to a higher molecular weight. Chitosan is very difficult to dissolve in water, alkaline solutions or common organic solvents. This is due to the formation of intermolecular hydrogen bonds of its molecules. However, it is soluble to some extent in dilute aqueous acid solutions. This is mainly due to the presence of amino groups in its molecular structure which get protonated in the aqueous acid solution rendering it soluble [16]. Thus, in the preparation of a solution of chitosan, an aqueous organic acid is always used as solubilising agent. The level of solubility of chitosan in dilute acids depends on its molecular weight and DD.

Viscosity is an important factor in the conventional determination of molecular weight of chitosan and its commercial applications. Chitosan viscosity is found to decrease with increased time of the demineralisation step in its preparation [17]. Bough et al. [18] found that in the treatment of chitin to make chitosan, deproteinisation with 3% NaOH and elimination of the demineralisation step decrease the viscosity of the final chitosan product. No et al. [19] demonstrated that chitosan viscosity is considerably affected by physical treatments (grinding, heating, autoclaving, ultrasonicating, but not freezing) and chemical treatments (e.g. ozone), wherein it decreases with an increase in treatment time and temperature. Kim et al. [20] noted a sharp decrease in chitosan viscosity in some organic acid solutions (40-60% in one day). However, the viscosity of chitosan solution stored at 4^{o}C was found to be relatively stable [19]. No et al. [21] reported a decrease in the viscosity of chitosan (1% chitosan in 1% acetic and/or lactic acid solution) with increased storage time and temperature. The decrease in viscosity recorded over time was related to the partial degradation of chitosan by the organic acid solutions. However, reports that relate the viscosity of chitosan to the type of solubilising organic acid solution are barely found in the literature [16].

A number of reports are found in the literature regarding conductivity studies of chitosan solutions [22-24], although this is not the case for the contact angle of chitosan solutions. Instead, reports are easily available for contact angles of liquids on films of pure chitosan or chitosan associated with other polymers [25-28].

It has been demonstrated that the type of acid used as a solvent of chitosan has an effect on the properties of chitosan [29-32]. This makes it necessary to understand the behaviour of the chitosan solution before using this polymer. The present work therefore reports on the investigation of the viscosity, contact angle and conductivity chitosan solubilised in dilute aqueous solutions of two organic acids (formic acid and acetic acid). A study of such kind is very essential before further exploring the compatibility of chitosan with other polymers in order to obtain blends for making films for various applications.

Materials and methods

Materials

Shrimp-derived chitosan sample with DD > 95% (defined by UV method [33]) was purchased from the chitin-chitosan laboratory at National University of Malaysia. Organic acids, viz. formic acid (98-100%, from Scharlau) and acetic acid (99.5%, from R & M Chemicals) were used as received. Distilled water was used to prepare all solutions. Freshly prepared solutions were used in all experiments.

Preparation of acid solutions of chitosan

A stock solution of chitosan was prepared by adding chitosan (0.1 g) to 100 mL of each acid solution (0.1 M) and heating the mixture at 40-50^{o}C with continuous stirring for 24 h. Each one of the solubilised solutions (0.1g/100mL) was then diluted to the desired concentration.

Methods

The molecular weight of chitosan was determined by gel permeation chromatography equipped with a Waters 1515 HPLC pump and a 2414 Refractive index detector. The column used was PL Aquagel-OH 30 (8 μm, 300 × 7.5 mm) and the solvent used was 1% acetic acid. The chromatograph was calibrated with polystyrene standards.

Viscosity measurements were conducted using a Ubbelohde-type viscometer (Fisher, Germany). The viscometer was connected to a visco-clock (Schott Visco clock) to record the time of solution passing through the two marks of the viscometer automatically. Every value recorded was an average of 4 measurements.

The relative viscosity, η_r, for a diluted polymer solution can be calculated from:

$$\eta_r = \frac{\eta}{\eta_o} = \frac{t}{t_o}$$

where η and η_o are the viscosities of polymer solution and the pure solvent respectively, t is the outflow time of the polymer solution and t_o is the outflow time for the pure solvent.

The specific viscosity, η_{sp}, was given by the relative increment in the viscosity of the polymer solution to the viscosity of the solvent as follows:

$$\eta_{sp} = \frac{(\eta - \eta_o)}{\eta_o} = \eta_r - 1$$

The reduced viscosity (viscosity number), η_{red}, is the specific viscosity per unit concentration C and was given by:

$$\eta_{red} = \frac{\eta_{sp}}{C} = \frac{(\eta_r - 1)}{C}$$

The intrinsic viscosity, $[\eta]$, can be determined from the reduced viscosity (η_{red}), which equals $\frac{\eta_{sp}}{C}$, or from the inherent viscosity, η_{inh}, which equals $\frac{\ln \eta_r}{C}$, by extrapolation to zero concentration [34]. To obtain an accurate value of the intrinsic viscosity of the chitosan solution, therefore, its average extrapolated value to $C = 0$ from the linear graphs of $\frac{\ln \eta_r}{C}$ and $\frac{\eta_{sp}}{C}$ versus concentration was taken.

The contact angle of the chitosan solution was measured using the contact angle measuring system G40 (Krüss gmbh., Hamburg, Germany) at room temperature. The measurement was done on microscope slides (25.4×76.2 mm, 1-1.2 mm thick). Each slide was cleaned before use by soaking in ethanol overnight. The average contact angle of both sides of the drop was taken into consideration. The average value of contact angle of water on the slide was 42^{o}. Sample volume used was 1 μL and the measurement time was 1.5 minutes.

Conductivity measurements were carried out using the Orion Model 105 conductivity metre. The actual conductivity reading was obtained by multiplying the observed conductance reading by the cell constant (K), which was equal to 1.0 $\mathrm{cm^{-1}}$ in this case. Calibration was done with standard potassium chloride solution. The equivalent conductivity is defined as:

$$\Lambda\ (\mathrm{ohm^{-1}\ m^{2}\ kg^{-1}}) = \frac{\mathrm{k\ (ohm^{-1}m^{-1})}}{\mathrm{C\ (kg\ m^{-3})}}$$

where Λ is equivalent conductivity, k is conductivity and C is concentration.

Results and Discussion

Determination of molecular weight

The molecular weight of chitosan used in this work was found to be 7.9 x 10^5 g $\mathrm{mol^{-1}}$. This value was somewhat higher than some of those reported in the literature. For example, it was higher than that reported by Ladet et al. (5.5×10^5 g $\mathrm{mol^{-1}}$) [35]. This is acceptable, however, since the molecular weight of chitosan depends on such factors as source of raw material and method of preparation.

Determination of viscosity

Figures 1 and 2 show the plots of $\frac{\ln \eta_r}{C}$ and $\frac{\eta_{sp}}{C}$ versus concentration for chitosan in the solubilising aqueous formic acid and acetic acid respectively. The two values of $[\eta]$ were obtained by linearly extrapolating the two graphs to $C = 0$ (Y-intercept). The data of intrinsic viscosity obtained are presented in Table 1, together with the values of coefficients $k_1$and k'_1, which were calculated from the slopes of the graphs. As can be seen, chitosan solubilised in acetic acid records a higher value of intrinsic viscosity. However, this value is lower than that in propionic acid reported by us earlier [36]. This behaviour may be related to the acid strength of the solubilising acid and the molecular volume of the solvated anion of chitosan.

It can also be observed that the intrinsic viscosity of chitosan in formic or acetic acid increased with the increase in the concentration of chitosan solution—a normal viscosity behaviour, in contrast to that determined in propionic acid [36]. Similar normal behaviour in the apparent viscosity of chitosan in aqueous organic acids was reported for pH varied between 2-5 [37]. Values of the coefficients k_1 and k'_1 were also found to be in agreement with those reported in the literature [38,39].

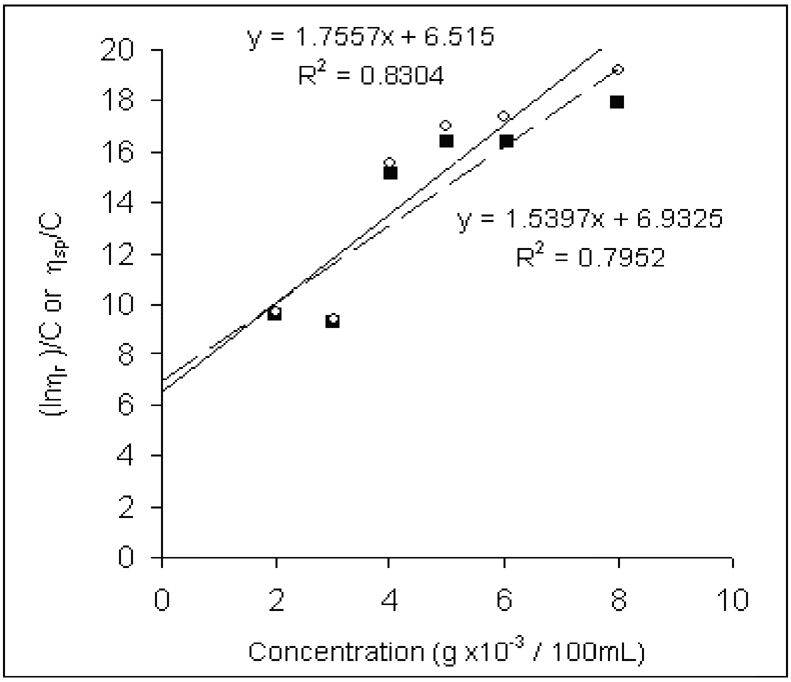

Figure 1. $\frac{\ln \eta_r}{C}$ (solid line) and $\frac{\eta_{sp}}{C}$ (dash line) of chitosan in formic acid solution versus concentration

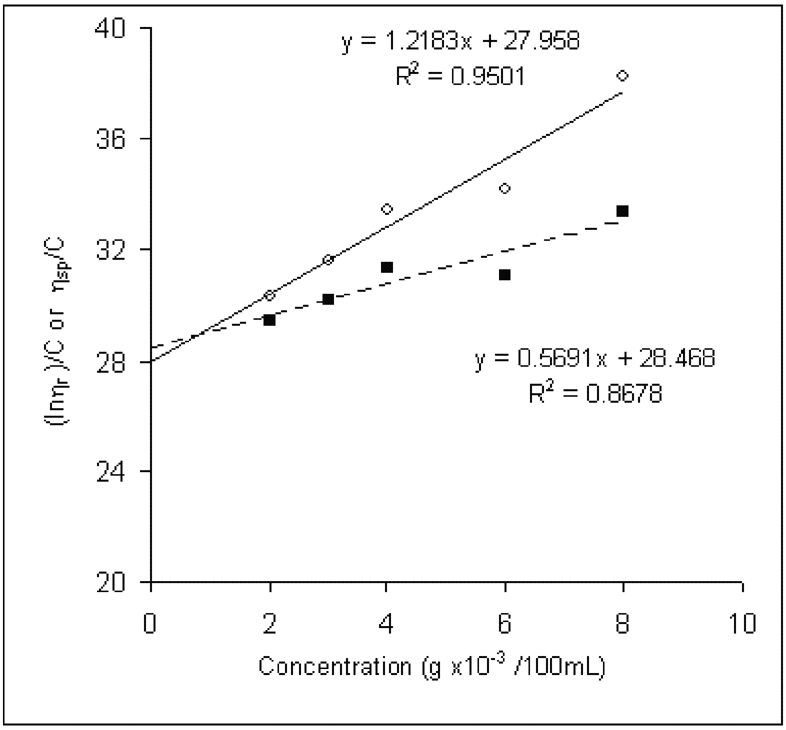

Figure 2. $\frac{\ln \eta_r}{C}$ (solid line) and $\frac{\eta_{sp}}{C}$ (dash line) of chitosan in acetic acid solution versus Concentration

Table 1. Values of $[\eta]$ ($cm^3\ g^{-1} x\ 10^2$), k_1 and k'_1 for chitosan in different solvents

Viscosity parameter	Formic acid	Acetic acid	Propionic acid*
$[\eta]$ (mean ± SD)	6.723 ± 0.294	28.213 ± 0.360	37.679 ± 0.340
k_1	0.032	0.702×10^{-3}	0.785×10^{-3}
k'_1	0.041	1.558×10^{-3}	0.487×10^{-3}

* Quoted from previous study [36] for comparison

Determination of contact angle

The average of contact angle is plotted as a function of concentration (Figures 3-4). Increase in the contact angle (decreas in $\cos\theta$) with increasing concentration of the solution may be linked to the departure from pure water (decrease in wettability) as the ratio of chitosan to water increases. However, a critical coagulation concentration (CCC) is reached, beyond which the contact angle becomes more or less independent of concentration [36,40]. The values of the CCC are about 9.5×10^{-5} and 7×10^{-5} g L^{-1} for chitosan in formic acid and acetic acid solutions respectively. These values are similar to that already reported for chitosan in propionic acid solution (4.5×10^{-5} g L^{-1}) [36].

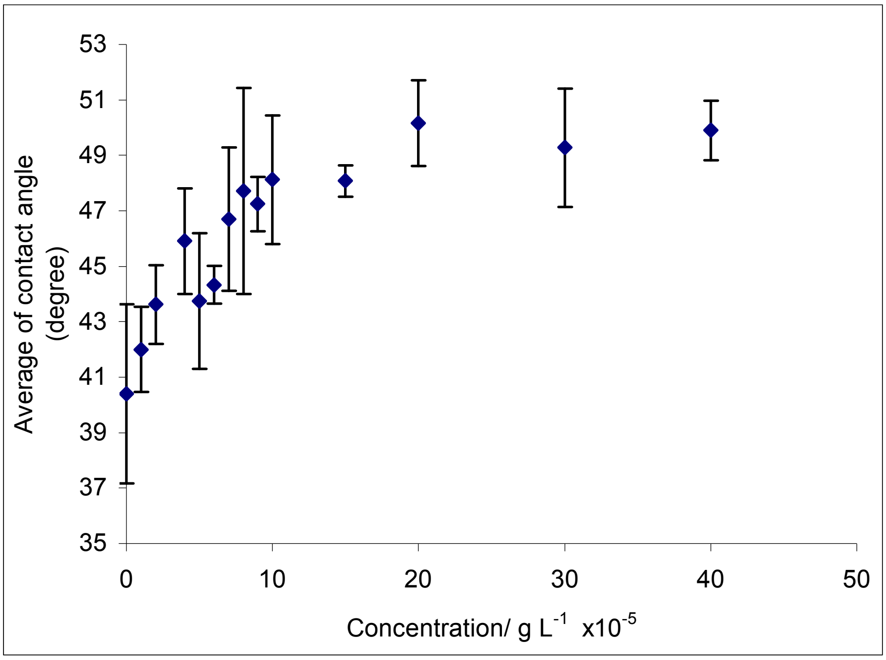

Figure 3. Contact angle of chitosan in 0.02 M formic acid solution versus concentration

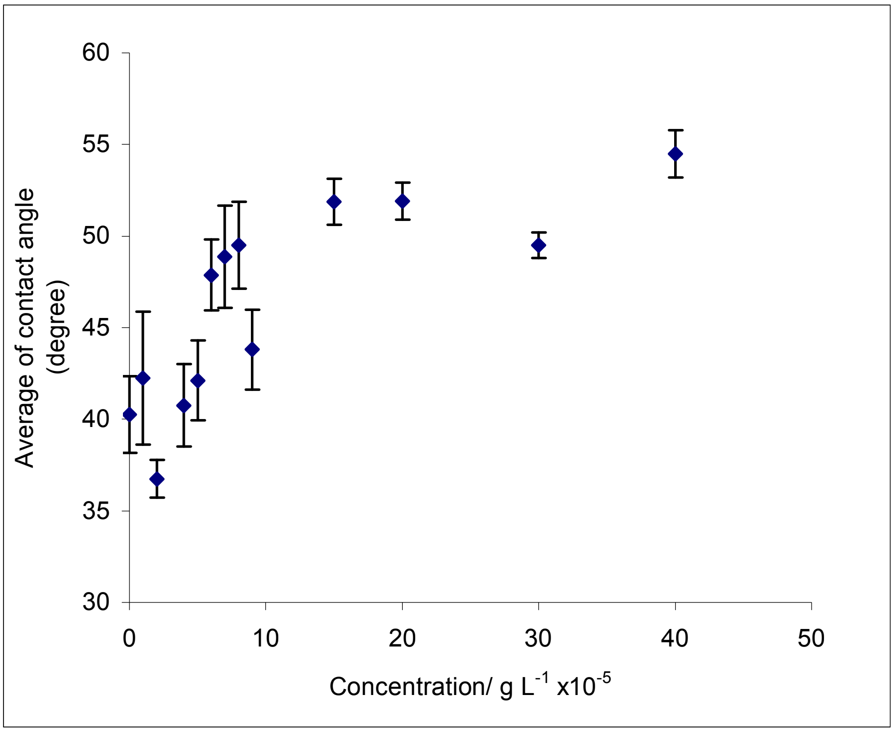

Figure 4. Contact angle of chitosan in 0.02 M acetic acid solution versus concentration

Determination of conductivity

Figures 5-6 show the correlation between equivalent conductivity and concentration. Equivalent conductivity decreases rapidly with increasing concentration until a CCC is reached, beyond which it decreases very gradually (almost constant)[36,41]. This behaviour is thought to be due to the decrease in ionic mobility of the polymer as concentration increases. Aggregation may have also occurred although probably not significantly. From this profile, the CCC values estimated (by noting a pronounced discontinuity in the curve) are 1×10^{-5} and 4×10^{-5} g L^{-1} for chitosan in 0.02 M formic acid and 0.02 M acetic acid respectively.

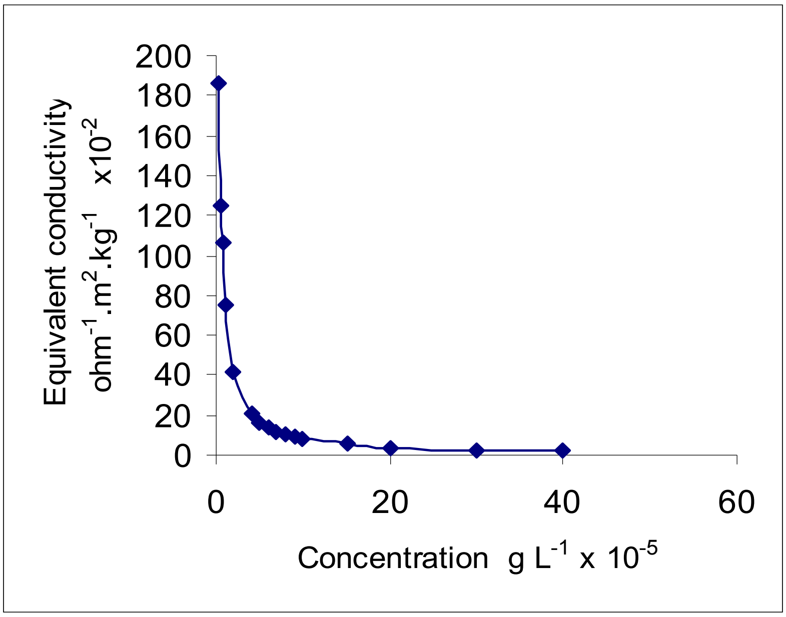

Figure 5. Equivalent conductivity of chitosan in 0.02 M formic acid solution versus concentration

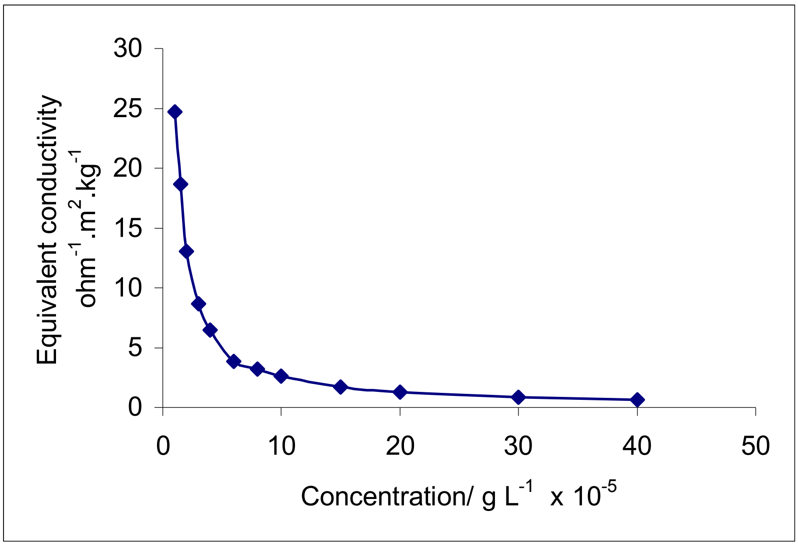

Figure 6. Equivalent conductivity of chitosan in 0.02 M acetic acid solution versus concentration

Conclusions

The intrinsic viscosity of chitosan with a molecular weight of 7.9 x 10^5 g mol^{-1} was 6.723 ± 0.294 and 28.213 ± 0.360 cm^3 g^{-1} in aqueous formic and acetic acids respectively. Contact angle of the chitosan solutions was found to increase initially with increase of the chitosan concentration up to a critical coagulation concentration (9.5×10^{-5} and 7×10^{-5} g L^{-1} in 0.02 M formic and acetic acids respectively). Conductivity of the chitosan solutions was found to decrease initially rapidly with increase of the chitosan concentration up to a critical coagulation concentration (1×10^{-5} and 4×10^{-5} g L^{-1} in 0.02 M formic acid and acetic acids respectively).

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